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Probing Behavior of *Aphis helianthi* (Homoptera: Aphididae) and Its Preference for *Pittosporum tobira* Leaves of Different Ages

G. P. WALKER

Department of Entomology, University of California, Riverside, California 92521 USA

Abstract.—A natural infestation of *Aphis helianthi* Monell exhibited a bimodal distribution on actively growing shoots of *Pittosporum tobira* Aiton. Peaks of abundance occurred on the youngest and oldest foliage. Aphids were also common on the mature leaves of year-old shoots that were producing new growth at their apices. Preference tests on leaf disks indicated a strong attraction to young leaves but not to mature or senescent leaves. Probing behavior was observed on young and mature leaves and duration of test probes was significantly longer on mature than on young leaves (median = 44.0s vs. 20.5s; $p = 0.0002$). The cuticle and outer epidermal cell walls of *P. tobira* were significantly thicker on mature than on young leaves (cuticle: 3.14μ vs. 1.08μ; cell walls: 6.60μ vs. 1.75μ; $p < 0.0001$). The results of the probing behavior experiments were not consistent with previous reports of probing behavior on *Citrus* and probable causes for the difference are discussed.

Although there have been many studies comparing aphid probing behavior among host and non-host or resistant and non-resistant plants, there have been few studies comparing probing behavior among different aged leaves of the same plant species. In one such study on *Citrus sinensis* (Linnaeus) Osbeck (Zettler et al. 1969), the duration of test probes by four species of aphids (*Aphis gossypii* Glover, *A. spiraecola* Patch, *A craccivora* Koch, and *Myzus persicae* [Sulzer]) was extremely brief on mature leaves (over 60% of the probes were only 1–15 sec. in duration) in contrast to longer probes on succulent, immature leaves. Because of the extreme brevity of these probes, the authors concluded that some quality of mature citrus cuticle and/or outer cell wall of the epidermis was repellent to probing aphids and that this quality was either chemical in nature or merely an impenetrability of the thick mature cuticle. This differs from the conventional explanation that leaf age preference by aphids is primarily a function of nutritional quality of phloem sap (Kennedy 1958). Since there is a paucity of probing behavior studies on leaves of different ages, it was of interest to determine if aphid preference of young over mature leaves could be associated with repellency by mature cuticle and/or outer epidermal cell walls in other plant species. This would be of particular interest in plants with traits similar to citrus that might be associated with thick, tough mature leaf cuticles: evergreen perennials with long-lived leaves that thrive in xeric conditions of southern California. *Pittosporum tobira* Aiton, a common ornamental plant in southern California, meets these criteria. An infestation of *Aphis helianthi* Monell on *P. tobira* in Riverside, California, provided an opportunity to determine 1) if this aphid exhibited leaf age preference on *P. tobira*; 2) if nonpreference of a leaf

age was associated with very brief test probes; and 3) if the duration of test probes was correlated with cuticle thickness.

Materials and Methods

Natural distribution.—A single *P. tobira* plant infested with *A. helianthi* was used for examining the distribution and leaf age preference of this aphid. This particular plant, unlike most ornamental *P. tobira,* was not shaped into a dense hedge and was therefore ideal for this study because it had long, actively growing terminals with a variety of leaf ages present on the same terminal. Effects of leaf age on the natural distribution of *A. helianthi* were evaluated during March 1985. Aphids were counted on the upper and lower surfaces from all leaves, from base to apex, on each of 6 actively growing terminals and each of 3 terminals from the previous year's growth. These 9 terminals comprised the bulk of the infestation on this plant.

Leaf age preference.—Aphids from this natural infestation were used to evaluate their leaf age preference. A cork borer (1.3 cm diameter) was used to cut leaf disks from leaves that were young (Y) (light green; not fully expanded; from near the apex of an actively growing terminal), mature (M) (fully expanded; dark green; hardened), or senescent (S) (mostly green but beginning to turn yellow on the upper leaf surface; lighter green on lower surface than mature leaves). Only one disk was cut per leaf. In each test, two leaf disks from each of two leaf ages were placed adaxial side down on moistened filter paper in a 5 cm diameter glass Petri dish. The 4-leaf disks were arranged in a square pattern so that leaf ages alternated and the edges of adjacent disks were in contact. Using an aspirator, 4 apterous aphids of various instars were carefully placed on each of the 4-leaf disks per Petri dish. There were four such dishes per test for a total of 64 aphids/test. The tests were conducted at room temperature and after 3.5–4.5 hrs. (except in one test: ca. 8.5 hrs.) numbers of aphids on each leaf disk were recorded.

Probe duration.—The durations of probes by *A. helianthi* on young and mature leaves were recorded using leaves collected from several field grown *P. tobira* plants. Leaves with intact petioles were collected by removing them from twigs and immediately immersing them in water. All tests were completed within 12 hrs. of picking the leaves. Leaves were placed individually in 1-dram shell vials filled with water and saturated cotton. The petioles were immersed in the saturated cotton and the blades extended erect above the vials. Parafilm was used to seal the opening of the vials around the protruding leaves such that the vials were water tight. The vials were held by a test-tube holder on a ring stand and could be easily rotated to facilitate microscopic viewing of the aphids on both leaf surfaces. Immediately prior to use, a thin barrier of petroleum jelly/mineral oil was placed on the base of the leaves to confine the aphids on the leaves.

Aphids were collected from a field plant in the morning (ca. 9–11 A.M.) and were held in glass Petri dishes without a food source until they were used in the experiment (two 10-min. periods between 1 P.M. and 10 P.M.). Using an aspirator, aphids (apterous adults and final nymphal instars) were carefully placed on the leaves and were observed for 10 min. with a Wild M5A stereomicroscope. A Volpi Intralux 5000 fiber optic ringlight provided a uniform and cool light source. The duration of each probe on the abaxial surface was recorded. If a probe started prior to the end of the 10-min. observation period and extended beyond it, the full duration of the probe

was recorded. The duration of a probe was defined as the time which the aphid was in characteristic "probing posture": labium extended perpendicular to the body plane and its tip touching the leaf surface. After the 10-min. testing period, each aphid was returned to a glass Petri dish without food. The next aphid was then tested on the alternate leaf age. After all aphids were tested once, each aphid was retested using a leaf age that was opposite to that used in its first test. There were 8 replicates, each conducted on a different day with different aphids. The first 4 replicates were conducted in April-May 1985 and the second 4 replicates in February-March 1986. The time involved in setting up the experiments resulted in only 5–9 aphids being used in any one replicate.

Duration of aphid probes (abaxial surfaces only) were analysed two ways. First, for each replicate, the duration of probes recorded on each leaf age was compared by the Mann-Whitney U test. Second, data from the 8 replicates were pooled and the median probe duration for each aphid on each leaf age was calculated. Median probe durations on young and mature leaves were paired for each aphid and were compared using the Fisher distribution-free sign test (Hollander and Wolfe 1973). Since test probes and not prolonged feeding probes were of major interest in the study, probe durations > 5 min. were truncated and given values of 5 min. prior to all analyses (such probes accounted for only 3% of all probes and had negligible effect on the medians).

Cuticle thickness.—Young and mature *P. tobira* leaves were collected for histological sectioning on 18, 19, and 23 April and 22 May 1985 (the same time period that replicates 1–4 of the probe duration experiment were conducted). These were fixed in FAA, embedded in paraffin, sectioned at 10μ, and stained with hemalum and safranin. The thickness of the cuticle and outer epidermal cell walls on the lower leaf surfaces were measured with an ocular micrometer at 1000X.

Results

Natural distribution.—On actively growing terminals, *A. helianthi* tended to be bimodally distributed with respect to leaf position (leaf age) on the terminals. Peaks of aphid abundance occurred at the basal (oldest) and/or apical (youngest) leaf positions. Fig. 1 illustrates the aphid distribution on an actively growing terminal where the bimodality was pronounced. The growth pattern of *P. tobira* is such that in the spring, groups of several new actively growing terminals tend to rise from the apex of a terminal from the previous year's growth. In addition to being present on actively growing terminals as described above, *A. helianthi* was also found in large numbers on some of the previous year's terminals that had actively growing terminals arising from their apices. The leaves on the previous year's terminals ranged from senescent at the base to mature at the apex. The limited number (3) of the previous year's terminals that were found infested with aphids prevents conclusions from being made, but it is important to note that aphids were found in large numbers on mature foliage of these terminals. In each of the 9 terminals that were examined, *A. helianthi* was found in greater numbers on lower leaf surfaces than on upper surfaces. In 7 of these 9 terminals the difference was significant ($p < 0.05$, Wilcoxon signed rank test).

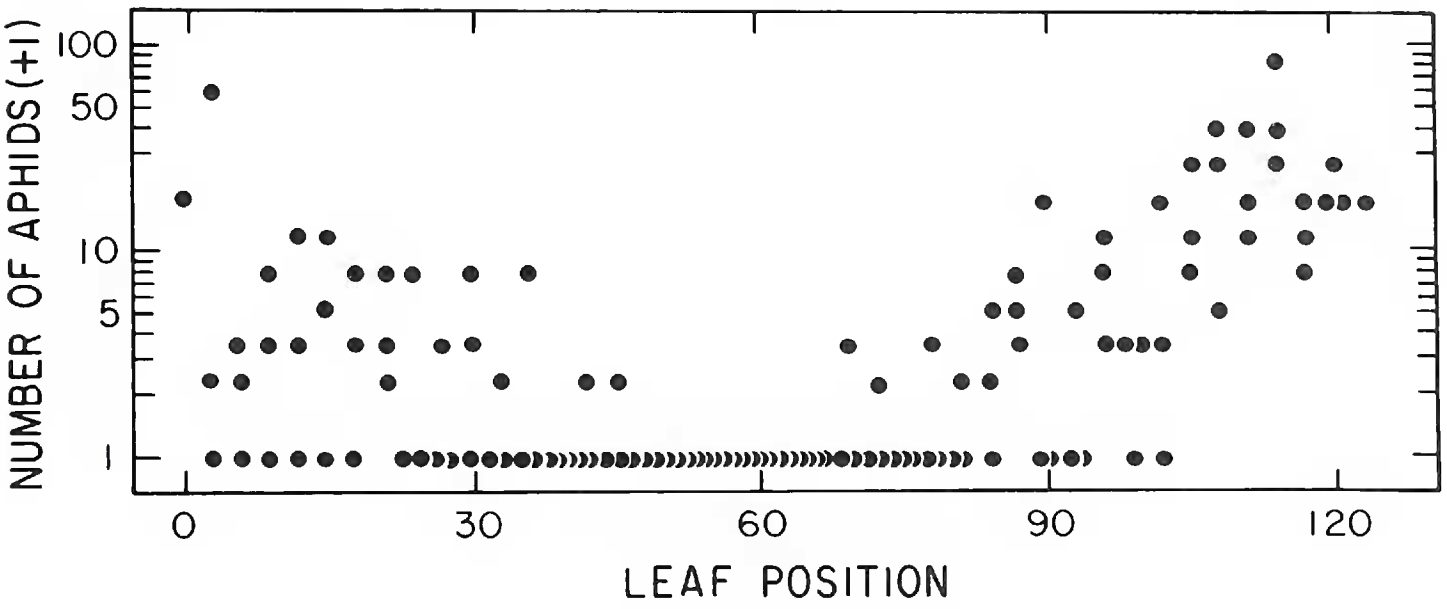

Figure 1. Numbers of *A. helianthi* found on leaves along the length of a long actively growing *P. tobira* terminal. Leaves are numbered from base to apex; number of aphids is plotted in a log scale (# aphids + 1).

Leaf age preference.—Table 1 shows that leaf disks cut from young leaves were preferred over those from mature or senescent leaves. In tests where no young leaf disks were included (M vs. S, Table 1), few aphids settled on any disk; most were on the moist filter paper or on the sides and top of the glass Petri dish. There was no consistent preference observed in the M vs. S tests although significantly more aphids were found on the mature disk than on the senescent disk after 4 hours in replicate 2 of the M vs. S comparison (Table 1). However, after 11 hours in this replicate there was no significant difference between the two ages of leaf disks (5 aphids were on each). The results presented in Table 1 did not significantly change over time in any of the other tests.

Probe duration.—Median probe duration of *A. helianthi* was greater on mature leaves than on young leaves in all 8 replicates (Table 2). The difference was significant at the $p = 0.10$ level in 7 of the 8 replicates and significant at the $p = 0.05$ level in 5 of the 8 replicates. When median probe durations on young and mature leaves were calculated for each aphid and analysed by paired analysis (pairing by aphid), probes on mature leaves were significantly longer ($p < 0.05$) than those on young leaves for the pooled data of each year of testing and the pooled data of both years combined (Table 2).

Cuticle thickness.—Histological examination of young and mature *P. tobira* leaves revealed that on lower leaf surfaces, the cuticle was significantly thicker on mature than on young leaves ($3.14\mu \pm 0.13$ vs. $1.08\mu \pm 0.20$; mean ± SE; $p < 0.0001$, t test). In addition, there was also a significantly thicker outer cell wall of the lower epidermal cells on mature leaves as compared to young leaves ($6.60\mu \pm 0.21$ vs. $1.75\mu \pm 0.47$; $p < 0.0001$, t test). The differences between lower leaf surfaces of young and mature leaves is illustrated in Fig. 2.

Table 1. *Aphis helianthi* preference for *Pittosporum* leaf disks cut from young (Y), mature (M), and senescent (S) leaves

Comparison	Leaf age	Number of settled aphids[a] Rep. 1	Rep. 2	Rep. 3
Y vs. M	Y	42	36	29
		***	***	***
	M	5	3	2
Y vs. S	Y	42	48	–
		***	***	–
	S	8	6	–
M vs. S	M	9	15	–
		n.s.	*	–
	S	6	4	–

[a]Total of 64 aphids (assorted instars) in each replicate; only those that settled on a leaf disk were used for analysis. Significance levels based on the binomial distribution and $H_o = P_o = 0.5$ are: *** $p < 0.0002$; * $0.01 < p < 0.005$; n.s. not significant.

Table 2. Median probe duration of *A. helianthi* on young and mature leaves of *P. tobira*.

	Median probe duration (sec.) and (N)[a] Young leaf	Mature leaf	Significance level
Replicate 1	20.0 (22)	54.0 (25)	.0087[b]
Replicate 2	16.0 (26)	40.0 (23)	.0003[b]
Replicate 3	32.0 (43)	48.5 (32)	.0002[b]
Replicate 4	24.5 (32)	39.0 (30)	.0275[b]
Pooled rep. 1–4 (1985 tests)	23.5 (29)	52.0 (29)	.0207[c]
Replicate 5	22.0 (29)	23.5 (26)	.9194[b]
Replicate 6	14.5 (34)	35.0 (14)	.0001[b]
Replicate 7	18.5 (46)	36.0 (30)	.0607[b]
Replicate 8	23.0 (43)	42.0 (36)	.0982[b]
Pooled rep. 5–8 (1986 tests)	19.0 (31)	35.0 (31)	.0011[c]
Pooled rep. 1–8	20.5 (60)	44.0 (60)	.0002[c]

[a]N = number of probes for Mann-Whitney comparisons and N = number of aphids for Fisher distribution-free paired comparisons.

[b]Mann-Whitney U-test.

[c]Fisher distribution-free sign test.

Discussion

Natural distribution.—The bimodal distribution of *A. helianthi* on actively growing terminals of *P. tobira* (Fig. 1) was not unexpected since this distribution pattern is commonly seen in the Aphididae as a group (Kennedy 1958). However, it was unexpected to find large numbers of aphids on mature leaves from some of the

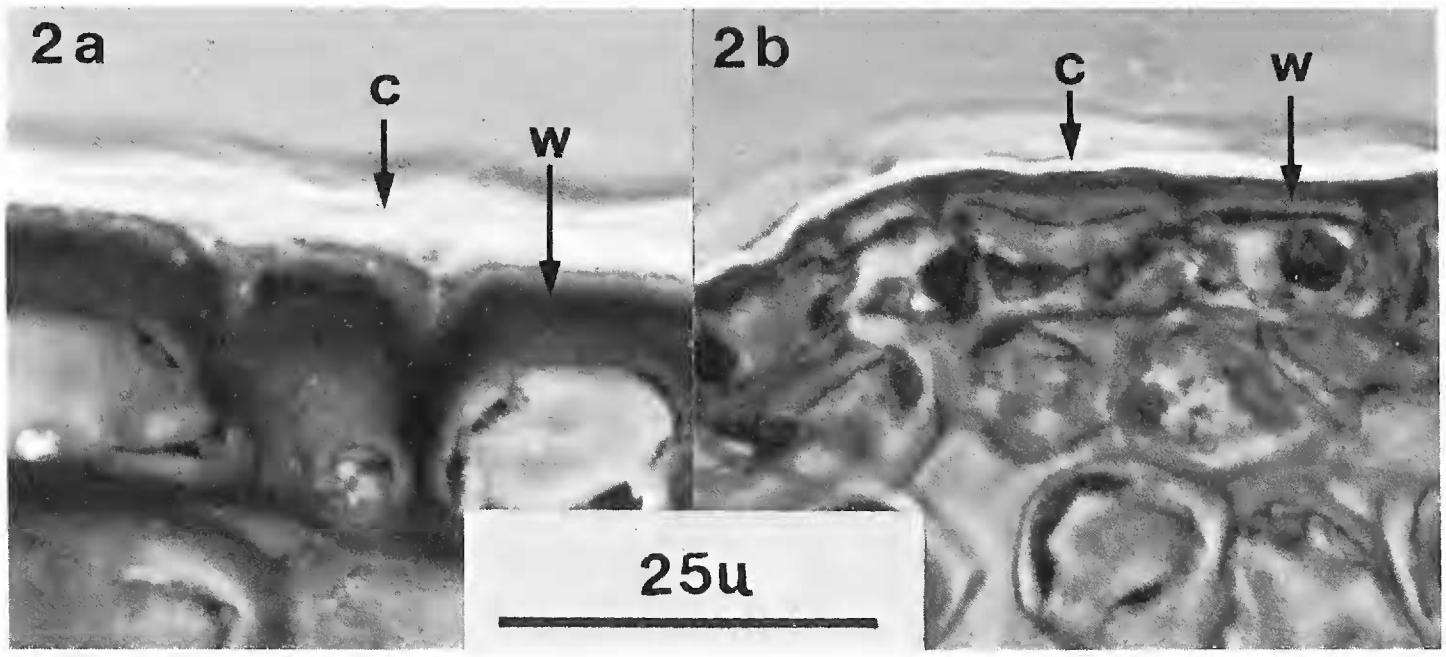

Figure 2. Lower leaf surface of *P. tobira* leaves. A: mature leaf. B: young leaf. c: cuticle. w: cell wall.

previous year's terminals that bore new, actively growing terminals at their apices. Perhaps these leaves were mobilizing nitrogen to supply the new, growing terminals and were consequently more nutritious for the aphids.

Leaf age preference.—*A. helianthi* showed a distinct preference for leaf disks cut from young *P. tobira* leaves vs. mature or senescent leaf disks (Table 1). This finding offers at least partial explanation for the great abundance of *A. helianthi* observed on young *P. tobira* leaves in nature (Fig. 1). Aphids did not readily settle on senescent leaves even though aphid abundance appeared to increase on senescent leaves in the field. However, Kennedy and Booth (1951) demonstrated that as leaves progress from the beginning of senescence to complete senescence, the preference of aphids for these leaves changes. Thus, the failure of the preference tests to detect preference of senescent over mature leaves may be a result of my criterion for choosing senescent leaves (slightly yellowed) not corresponding to the criterion that aphids presumably was (high amino acid concentration in the phloem). Alternatively, preferences observed on intact plants are not always detectable when using excised plant parts (Risch 1985) and may explain the discrepancy between the natural distribution and the preference tests. Nonetheless, both the natural distribution and preference tests suggested preference for young over mature leaves. Therefore, the probing behavior was studied in detail on these two age classes.

Probe duration and cuticle thickness.—The duration of probes by *A. helianthi* was significantly greater on mature *P. tobira* leaves than on young leaves. In contrast, Zettler et al. (1969), observed that probe duration of four species of aphids on *Citrus sinensis* was shorter on mature leaves than on young leaves. It is important to emphasize that most of the probes in both this study and in Zettler et al. (1969) were "test probes" and not phloem feeding probes since most were less than one minute and aphids require approximately a minute to penetrate the epidermis and considerably longer to reach the phloem (Pollard 1977). Both plant species are characterized by possessing a thin immature leaf cuticle and a thick mature leaf cuticle (*P. tobira*: Fig. 2, *Citrus sinensis*: Walker, unpublished observations).

Therefore, the mere greater thickness of mature cuticle compared to immature cuticle is not likely to be, by itself, the cause for the extremely brief probes observed on mature *Citrus*. This contention also is supported by Zettler et al. (1969) who observed that fully expanded *Crotalaria spectabilis* Roth leaves (which had cuticles similar in thickness to mature *Citrus* leaves) did not elicit the large proportion of brief probes seen on mature *Citrus* leaves. They did not compare young and mature *Crotalaria* leaves.

Clearly, the process of discrimination between young and mature leaves differs between aphids feeding on *P. tobira* and those on *C. sinensis*. I hypothesize that A. *helianthi* does not discriminate between young and mature *P. tobira* leaves until its stylets penetrate the cuticle and it can sample the internal constituents of the leaf, and that the greater duration of test probes on mature leaves is a consequence of the extra time required to penetrate the extremely thick mature cuticle and outer epidermal cell wall. This hypothesis is congruous with the widely held belief that aphid leaf age preference is primarily a function of the nutritional quality of the leaf. However, in the case of citrus, aphids probably discriminate between young and mature leaves before their stylets penetrate the cuticle and hence mature leaves are rejected after very brief test probes. In citrus, the epicuticular wax or other surface components may contain probing repellents or lack required probing stimulants (Klingauf et al. 1978, Jördens-Röttger 1979).

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